SENSORY EPITHELIUM OF PHARYNX AND CILIATED PITS OF MICROSTOMA CAUDATUM.

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For more than four years the senior writer has been able to collect *Microstoma caudatum* Ldy. from a little ice pond at the eastern border of Charlottesville, Va. These animals during the late autumn and on into early winter are frequently found sexually mature; all that we have found in such condition proved to be females. A mature female is indicated in our first figure, plate I. No males have been recognized by either of us. Many specimens have been sectioned and their sections studied in series. These studies have shown that the sexually mature individuals were not hermaphroditic.

Specimens have been fixed in hot aceto-sublimate, Bouin's fluid, and chrom-aceto-formalin mixture. In all cases the last medium has given us the best results. We think it highly important that the specimens be handled individually in fixing. Our studies of the histology of the ciliated pits have been based upon material fixed in chrom-aceto-formalin for twenty-five minutes. Specimens were then rinsed in two or three changes of tap water and carried through to paraffin. Sections were made from three micra to ten micra thick. Iron hæmatoxylin with Bordeau red as a counter stain was employed.

Microstoma caudatum Ldy. has a spindle-shaped body measuring from 750 micra to 1.5 millimeters in length. Its anterior end is more rounded than the pointed, posterior end. The entire surface is highly ciliated. The mouth, leading into a conspicuous, ciliated pharynx or œsophagus, lies on the ventral side about one sixth of the length of the body posterior to the anterior end. No eyes or "eyes-spots" have been observed by us. The ciliated pits lie dorsal to the mid-lateral surface of the body a little anterior to the mouth. These organs together with the pharynx can be closed or distended. When the animal is testing

the water the ciliated pits and the pharynx are opened and closed in such a manner as to greatly alter the contour of the anterior end. These then constitute invaginated regions of the body-surface which test the character of the water passing over the body and into the pharynx. This being the case it is of interest to see the relation of the central nervous system to the epithelia of the pharynx and of the ciliated pits.

The central nervous system of *Microstoma caudatum* Ldy. consists of two anterior ganglia connected by a very short, wide, transverse commissure. Extending from each ganglion is a dorsal, lateral nerve and a ventral, lateral nerve. The ventral,

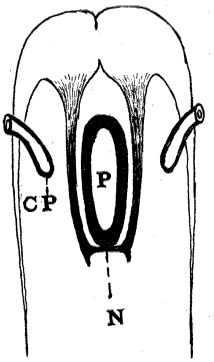

FIG. 1.

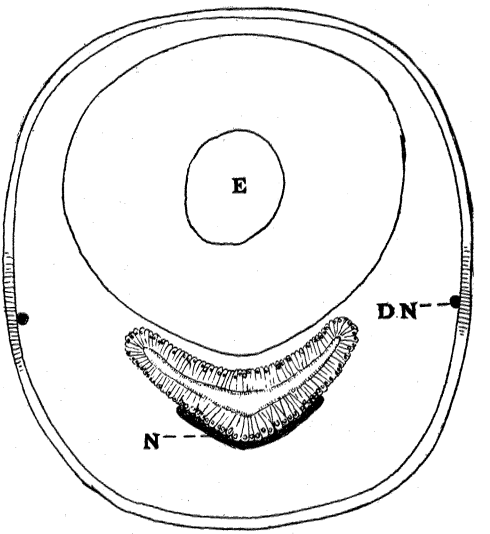

FIG. 2.

FIG. 1. Diagram of central nervous system showing its relation to ciliated pits and pharynx. *CP*, ciliated pit lying over dorsal, lateral nerve; *P*, pharynx; *N*, posterior commissure connecting the two ventral, lateral nerves.

FIG. 2. Diagram of a transverse section of *Microstoma* to show the position of the gustatory epithelium and the posterior nerve commissure. *N*, posterior nerve commissure lying in contact with the gustatory epithelium of pharynx; *DN*, dorsal, lateral nerve; *E*, lumen of enteron.

lateral nerves are connected by a commissure just posterior to the pharynx (text-figures 1 and 2, *N*). In the posterior floor of the pharynx there is a transverse region of its ciliated epithelium, which, so far as we can make out, is free from any ducts of

the unicellular glands of the pharynx, but which has its cells resting directly upon a transverse commissure connecting the ventral, lateral nerves (text-figures 1 and 2, *N* and Fig. 3, *N*). Martin ('08, Fig. 10) has shown how the pharynx of *Microstoma caudatum* Ldy. is everted when about to ingest a *Hydra*. In a similar way we have seen *Microstoma caudatum* Ldy. evert its pharynx in testing the edible or non-edible quality of plants and debris about which it was swimming. Judging from the intimate morphological relation between this band of pharyngeal cells and the nerve commissure and the manner in which the pharynx is operated, we believe we have here a *primitive gustatory organ.*

The ventral nerves in passing along the right and left sides of the pharynx might have made similar contacts with the pharyngeal epithelium and thus the gustatory organs would have been in this case bilaterally arranged, but in such case they would not be directed at right angles to what is the usual source of stimulus due to the animal moving anteriorly and to the food falling upon the floor of the pharynx. Thus we find this very primitive gustatory organ, in a free-moving bilaterally symmetrical animal, to be a median structure.

The ciliated pit when closed is a club-shaped sac which rides the dorsal, lateral nerves (text-figure 1, *CP*). Its lumen is lined with cilia much stronger than the cilia of the general surface. These are especially heavy near the mouth of the pit. The ciliated columnar cells of the ventral side near the mouth lie in intimate contact with the dorsal, lateral nerve (Fig. 4, *DN*). Hence we consider them to be the peculiar sensory cells of the ciliated pit, though we can see no other striking difference between them and the other ciliated cells of the outer half of the pit. Men have always looked upon these structures as being organs of special sense. It is important to state in this connection that here we have the same habit as the pharynx displays of repeatedly exposing the sensory cells to contact with stimuli by opening and closing the pit and the same kind of nerve supply as we have in the median gustatory epithelium of the pharynx. Their reason for being bilaterally placed is suggested in the latter part of this paper.

The epithelium of the ciliated pit, just as that of the pharynx,

represents a modified region of the general body-epithelium. This differentiation can be studied in specimens undergoing binary fission. Fig. 2 represents a ciliated pit in a newly-forming individual. In this it is shown that as the flat, pavement epithelial cells of the general surface pass into the wall of the embryonic pit they become taller until at the fundus or blind end of the pit there are very tall, columnar cells. As differentiation proceeds, however, we get a point of great systematic interest that has not been made so far as we have been able to determine. The cells at the fundus of the growing pit continue to grow and differentiate themselves as unicellular glands. Thus in the fully developed pit we have its fundus composed of large, glandular cells which lie deeply embedded in the mesoderm. These cells have pear-shaped bodies measuring 10 micra to 12 micra in diameter. Each cell has a glandular duct leading into the lumen of the pit (Fig. 4, *G'*). It is not therefore as von Graff ('09, Seit 64) says of rhabdocœles that "Die Grübchenflecken sind Hautstellen, die keine Rabdoide und Drüsenausführungsgänge besitzen"; nor is the histology of Wilhelmi's "Auricularsinnesorgane" described as being so differentiated. In the mature ciliated pit of *Microstoma caudatum* Ldy. we have a differentiation of its cells into a sensory and a glandular region (Fig. 4). The apparent cuticula shown in this figure is but the cut ends of cells radiating from the plane of the section, and it cannot be considered analogous to the "homogeneous mass" which Ott ('92) found covering the ciliated ends of the cells in the ciliated pit of *Stenostoma leucops* O. Schm.

As stated above this has considerable systematic importance. Zoölogists look upon the affinity between Turbellaria and Nemertini as being very strong. The "cerebral organs" of Nemertini have been considered the homologues of the ciliated pits of Turbellaria. These "cerebral organs" in the highest Nemertini show no resemblance to the ciliated pits of any turbellarian but the "cerebral organ" "in its simplest form, in the Protonemertini, is a mere groove in the epidermis not extending deeper than the basement membrane; it is lined by ciliated cells, and at the bottom are large nerves from the brain" (Benham, '01, p. 185). So it appeared that the simplest "cerebral

organs" differed from ciliated pits only in that the former were differentiated into a glandular region and a sensory region. In *Microstoma caudatum* Ldy. we find a similar differentiation into glandular and sensory regions. Thus we see the affinity between Turbellaria and Nemertini strengthened by the structure of the ciliated pits of *Microstoma caudatum* Ldy.

So much for the morphological part of the present paper. The second part has to do with experiments performed upon *Microstoma*.

We have collected *Microstomas* by placing into an aquarium sticks, leaves, and other submerged objects that have been taken from the marginal bottom of the pond. It is only after the filled aquarium, containing the debris from the pond, has stood twelve to twenty hours that the *Microstomas* appear at the surface of the water. In making collections for histological material it was noticed that the animals were quite alert and active when they first appeared at the surface. Under laboratory conditions bacteria accumulate rapidly in the aquaria so that within forty-eight hours a thin film appears at the surface. After this film has appeared the *Microstomas* seem to be less alert, and when this film has become dense the *Microstomas* must be actually touched in order to be caused to move from the place in which they were lying. This observation was made only after a point brought out by some of the experiments, to be described below, had indicated it.

If specimens are collected as soon as they appear at the surface and studied under supported cover-glasses by means of the compound microscope they are seen to make numerous exploratory movements as they swim about by thrusting their anterior ends to and fro, thus testing the water by means of their ciliated pits and pharynx. These actions then may be taken to indicate the nearly normal response of *Microstoma* to nearly normal conditions.

Next some specimens were placed in a .05 per cent. salt solution to see what their response would be to less normal conditions. It was found that the exploratory movements were intensified and the ciliated pits widely distended with each anterior thrust of the body. Thus it was seen that a change in conduct could be induced by artificial media.

In casting about for reagents with which to further experiment we decided to try dilute acetic acid because these specimens were found among decaying vegetable matter in which we inferred acetic acid might be present. Specimens were placed in eosin water.[1] One of these in eosin water was placed under a supported rectangular cover-glass and by means of a capillary pipette, mechanically controlled, a drop of 1/25 per cent. acetic acid added. The specimen swam about until it came in contact with the colorless margin of the drop of acetic acid solution when it reacted by turning directly away from the acid as indicated by text-figure 3. This experiment was repeated

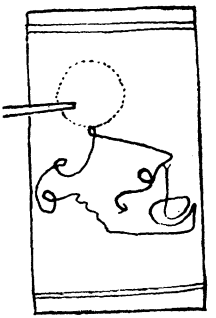

FIG. 3.

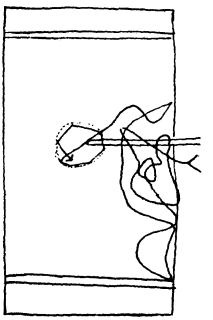

FIG. 4.

FIG. 3. Diagram indicating the path of a specimen when placed in eosin water and encountering a drop of 1/25 per cent. acetic acid solution. The dotted line indicates the contour of the enclosed drop of 1/25 per cent. acetic acid surrounded by eosin water.

FIG. 4. Diagram indicating the path of a specimen when placed in .1 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the enclosed drop of fresh water surrounded by .1 per cent. salt solution.

eighteen times with practically the same results. When the animals came near the zone of acetic acid their anterior ends would be expanded to such a great extent as to make them distinctly three-lobed. When the acid was reached the anterior end would be greatly distorted while the posterior end would show no change of shape. Occasionally a specimen would strike the acid with such momentum that it would be carried into it. When they had thus entered the acid, globules would appear about the sides of the anterior end as if the ciliated pits were discharging droplets of mucus. When such specimens escaped from the

[1] Fresh water in which just enough eosin was dissolved to make it distinguishable from the acid solution.

acid they ceased to show any exploratory movements. Perhaps this loss of the exploratory movements may be accounted for by injury of the ciliated pits. Usually the specimens would be unable to free themselves from the acid and would die. A 1/50 per cent. acetic acid solution was tried with similar results.

Thus it is seen that both 1/25 per cent. or 1/50 per cent. acetic acid are dangerous—even fatal media to *Microstoma*. Therefore .1 per cent. and .05 per cent. common salt solutions were tried. The first experiment was made upon an animal *seventeen hours* after removal from the pond. The animal was placed in a .1 per cent. salt solution and left to find the drop of fresh water which was placed in the center. The animal's course is indicated in text-figure 4. When the animal entered the fresh water it continued straight across it until it came to the far side of the drop, then it would turn away from the ciliated pit lying nearest the salt solution. By repeating this reaction to contacts with the salt solution the specimen would rotate in the drop of fresh water.

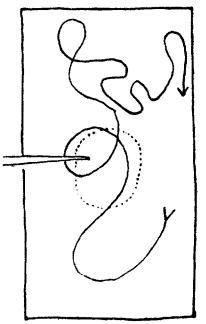

FIG. 5. Diagram indicating the path of a specimen when placed in .1 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the enclosed drop of fresh water surrounded by .1 per cent. salt solution.

We made only one experiment within twenty-four hours after the specimens were removed from the pond. *Five days later* specimens were taken from the same aquarium which furnished the last one. These specimens showed no definite reaction to the fresh water when they were treated like the specimen referred to in text-figure 4. The course of one of these is shown in text-

figure 5. It can be seen that the animal passed through the fresh water twice and did not react to it at all.

These experiments suggested to us that a change may have taken place in the physiological condition of *Microstomas* which were kept in the laboratory for a certain length of time.

Collections were then made of *Microstomas* and 150 additional experiments performed during five months in order to test the suggestion made by these two sets of experiments.

These experiments must be divided into two groups and each of these groups into two sub-groups:

1. Reactions of *Microstomas* kept in the laboratory for less than twenty-four hours.

(*a*) When they were placed in fresh water and left to come in contact with an undisturbed drop of .05 per cent. common salt solution which was placed in the center.

(*b*) When they were placed in .05 per cent. salt solution and left to come in contact with an undisturbed drop of fresh water which was placed in the center.

2. Reactions of *Microstomas* kept in the laboratory for more than twenty-four hours.

(*a*) When they were placed in fresh water and left to come in contact with an undisturbed drop of .05 per cent. common salt solution which was placed in the center.

(*b*) When they were placed in .05 per cent. salt solution and left to come in contact with an undisturbed drop of fresh water which was placed in the center.

The following are three experiments of each of the above groups picked at random from our notebooks:

Group 1-*a* (specimens placed in fresh water and allowed to encounter a drop of .05 per cent. salt solution).

January 13, 1912. A specimen that had been in the laboratory *twenty hours* came in contact with the drop of .05 per cent. salt solution and each time reacted by turning away from the margin of the salt solution (text-figure 6-*a*).

February 3, 1912. A specimen that had been in the laboratory *eighteen hours* came in contact with the drop of .05 per cent. salt solution five times and each time it reacted by turning away from the margin of the salt solution (text-figure 6-*b*).

November 30, 1911. A specimen that had been in the laboratory *nineteen hours* came in contact with the drop of .05 per cent. salt solution six times and each time it reacted by turning away from the margin of the salt solution (text-figure 6-*c*).

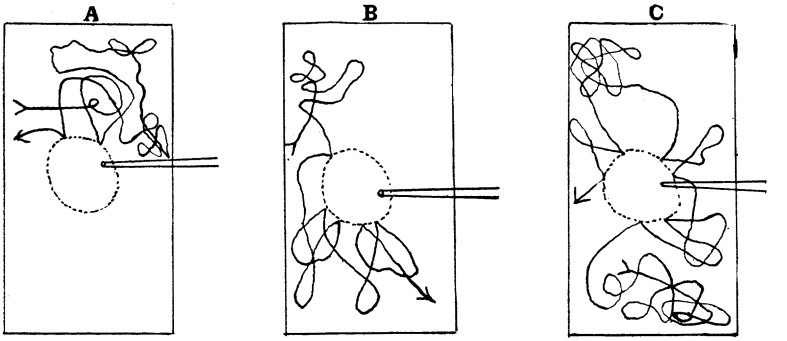

FIG. 6. Diagrams indicating the paths of specimens when placed in fresh water and encountering a drop of .05 per cent. salt solution. The dotted line indicates the contour of the drop of .05 per cent. salt solution surrounded by fresh water.

None of these specimens so treated entered the salt solution.

Group 1-*b* (specimens placed in .05 per cent. salt solution and allowed to encounter a drop of fresh water).

January 9, 1912. A specimen that had been in the laboratory *seventeen hours*. This one was caught in the drop of fresh water as the water was issuing from the pipette. Although it came in contact with the margin of the salt solution six times it did not leave the fresh water as shown by text-figure 7-*a*.

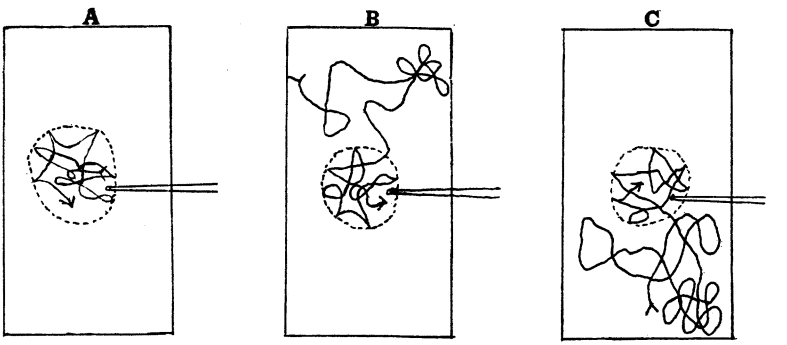

FIG. 7. Diagrams indicating the paths of specimens when placed in .05 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the drop of fresh water surrounded by .05 per cent. salt solution.

December 2, 1911. A specimen that had been kept in the laboratory *twenty hours* after entering the drop of fresh water did not leave it although it came in contact with the margin of the salt solution six times (text-figure 7-*b*).

January 13, 1912. A specimen that had been kept in the laboratory *twenty-four* hours after entering the drop of fresh water did not leave it although it came in contact with the salt solution six times (text-figure 7-*c*).

None of these specimens after entering the fresh water left it.

Group 2-*a* (specimens placed in fresh water and allowed to encounter a drop of .05 per cent. salt solution).

November 22, 1911. A specimen that had been kept in the laboratory *five days* passed five times from the fresh water into the salt solution and did not show any reaction to the latter at all (text-figure 8-*a*).

November 20, 1911. A specimen that had been kept in the laboratory *six days* passed from the fresh water into the salt solution three times and did not show any reaction to the latter (text-figure 8-*b*).

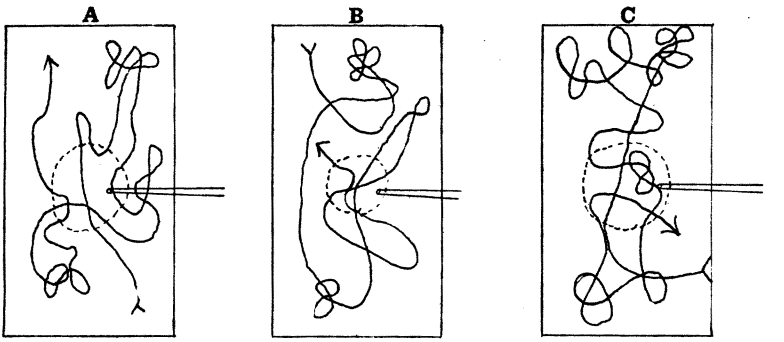

FIG. 8. Diagrams indicating the paths of specimens when placed in fresh water and encountering a drop of .05 per cent. salt solution. The dotted line indicates the contour of the drop of .05 per cent. salt solution surrounded by fresh water.

December 2, 1911. A specimen that had been kept in the laboratory *three days* passed from the fresh water into the salt solution three times and did not show any reaction to the latter (text-figure 8-*c*).

These specimens show a marked difference in their conduct as

compared with the specimens of Group 1-*a*, in that they *pass through the drop of .05 per cent. salt solution* instead of turning away from it.

Group 2-*b* (specimens placed in .05 per cent. salt solution and allowed to encounter a drop of fresh water).

December 16, 1911. A specimen that had been kept in the laboratory *three days* entered the fresh water three times but each time it left it without showing any reaction to the salt solution (text-figure 9-*a*).

January 9, 1912. A specimen that had been in the laboratory *four days* entered the fresh water three times but each time it left it (text-figure 9-*b*).

November 20, 1911. A specimen that had been in the laboratory *five days* entered the fresh water three times but each time left it (text-figure 9-*c*).

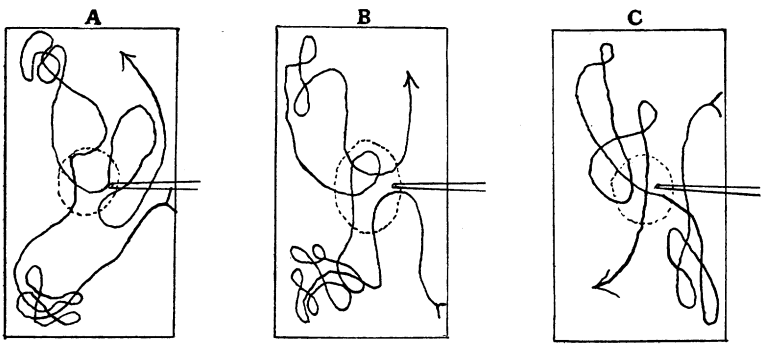

FIG. 9. Diagrams indicating the paths of specimens when placed in .05 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the drop of fresh water surrounded by .05 per cent. salt solution.

These specimens, too, show a marked contrast with Group 1-*b* in that *they pass indifferently from .05 per cent. salt solution into fresh water and back into the .05 per cent. salt solution* instead of remaining in the drop of fresh water.

Thus our experiments have sustained the inference that the physiological condition of *Microstoma* is lowered by the conditions peculiar to laboratory aquaria. This loss of physiological tone generally takes place after the first twenty-four hours.

The conditions most strikingly peculiar to laboratory aquaria

are: (*a*) Radical changes of temperature; (*b*) rapid accumulation of bacteria. That the first is not the greater factor is shown by the fact that in one aquarium, in which for some reason bacteria did not accumulate, specimens remained from December 8 until February 3 and still gave reactions which showed that they had not lost their physiological tone. Text-figure 10-*a* indicates the path of such a specimen when placed in fresh pond water containing near its center a drop of eosin .05 per cent. salt solution. It would be well to compare this figure with text-figure 10-*b* which indicates the path of a specimen, with reference to a drop of .05 per cent. salt solution, which had been kept in an aquarium only five days, but upon which vessel a thin glea of bacteria had collected. As can be seen the latter specimen was indifferent to the salt solution.

This observation led us to test the reactions of two lots of specimens taken from fresh aquaria:

The first lot we placed in a watch glass with some of the brown glea of an old aquarium for two hours. These specimens showed plainly that they had lost their physiological tone. Text-figure 11-*a* indicates the path of one of these specimens when placed in .05 per cent. salt solution containing a drop of fresh water in

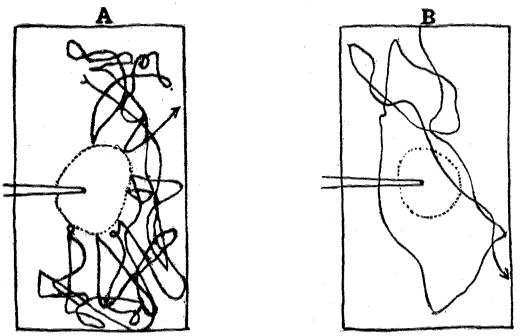

FIG. 10. Diagrams indicating the paths of specimens when placed in fresh wate and encountering a drop of .05 per cent. salt solution. The dotted line indicates the contour of the drop of .05 per cent. salt solution surrounded by fresh water.

the center. It can be seen that the specimen passed from one medium into the other six times and did not react to them at all.

The second lot we placed in a watch glass and then put this

on top of a paraffin bath which kept the water at a constant temperature of 86° F. Although these specimens were kept at this high temperature for twenty-four hours they gave us reactions which showed that they had not lost their physiological tone.

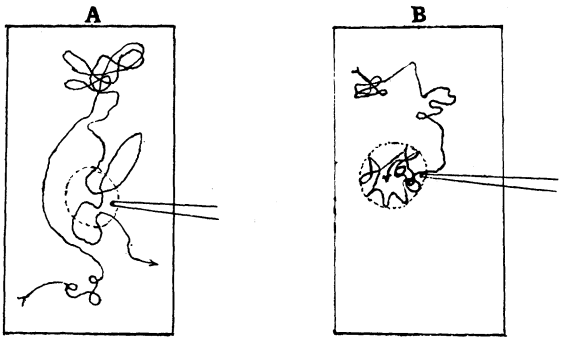

FIG. 11. Diagrams indicating the paths of specimens when placed in .05 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the fresh water surrounded by .05 per cent. salt solution.

Text-figure 11-*b* shows the path of one of these when treated like the one placed in bacteria. As soon as this specimen entered the fresh water it remained there by continually reacting to the salt solution. We watched such a specimen remain in the drop of fresh water for thirty minutes and then stopped observations because the salt solution began to diffuse into the fresh water. A comparison of text-figures 11-*a* and 11-*b* shows the difference between the reactions of the above two lots of specimens.

From these experiments we conclude that:

(*a*) *Microstomas* lose their physiological tone chiefly from the toxins thrown off by the bacteria.

(*b*) If change of temperature is a factor in this loss of physiological tone it is a very small one and they in time become adjusted to the new factor in their environment.

This change of physiological condition is somewhat analogous to the different physiological states found in the flat-worm Planaria by Pearl ('03) and summarized by Jennings in his "Behavior of the Lower Organisms" (p. 253, '06).

Finally a second class of experiments was carried out in order to test the value of the bilateral arrangement of the ciliated pits.

With a fine knife, made from a flattened needle, the ciliated pit was cut from either side of the animal. When the right pit was removed the specimen moved about in a spiral path keeping the remaining or left ciliated pit directed towards the center of the spiral path. When the right pit was left intact and the left destroyed the spiral movement was in the opposite direction. Thus the bilateral disposition of the ciliated pits of *Microstoma* serves the purpose of orienting or directing the *Microstoma* in its course through the water.

Again both pits were removed. In such cases it sometimes happened that the cut was a clean one, leaving the specimen divided into a minute, anterior part bearing the ciliated pits and a large, posterior part lacking the ciliated pits and the "brain." In such a case the minute portion moved in a highly active manner, tumbling about in all directions, while the large portion moved slowly in a direct line, except that a wide arc to the right or left was occasionally made. This direct course was continued until some inert object was encountered. Such contact would cause a change in the path of the specimen. This large part displayed no exploratory movements. So it is further suggested that the exploratory movements of *Microstoma* depend upon the ciliated pits being in a functional condition.

Summary.

1. There is present in the mid-ventral floor of the pharynx of *Microstoma caudatum* Ldy. a sensory epithelium, free from gland-ducts, which lies directly in contact with the posterior, transverse nerve commissure. The manner in which the pharynx behaves in testing food suggests that this is an elementary gustatory epithelium.

2. The ciliated pit has a glandular and a sensory region. Thus it resembles the "cerebral organs" of the Protonemertini and strengthens the affinity between the Rhabdocœles and the Nemertini.

3. *Microstoma caudatum* Ldy. living in its normal medium tests the surrounding water, etc., with its pharynx and ciliated pits. This testing is facilitated by making numerous exploratory movements with its anterior end.

4. We can recognize two physiological conditions in *Microstoma caudatum* Ldy.: 1st, when it has its physiological tone; 2d, when it does not have its physiological tone. In the first case it can and does distinguish between its normal medium and an artificial one such as .05 per cent. salt solution. In the second case it does not make this distinction.

5. This loss of physiological tone under laboratory conditions is caused chiefly by the toxins thrown off by bacteria; if change of temperature is a factor in this loss, it is but a slight one and in time *Microstoma* adjusts itself to this change and regains its physiological tone.

6. The bilateral disposition of the ciliated pits serves to direct the animal in its movements.

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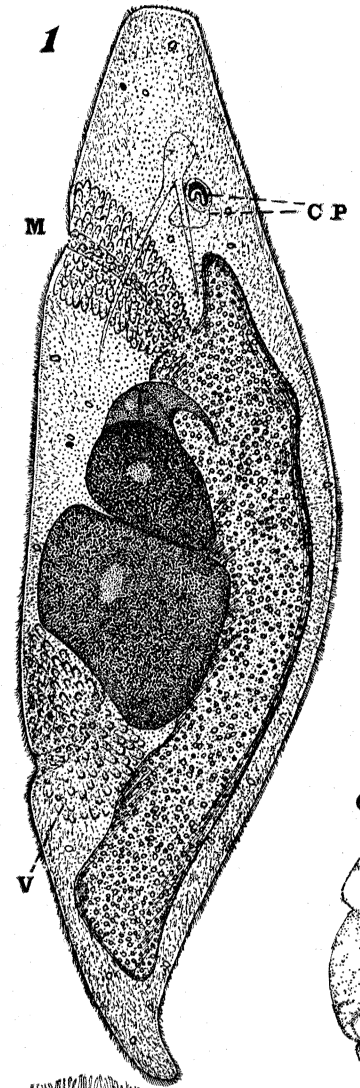
Explanation of Plate I.

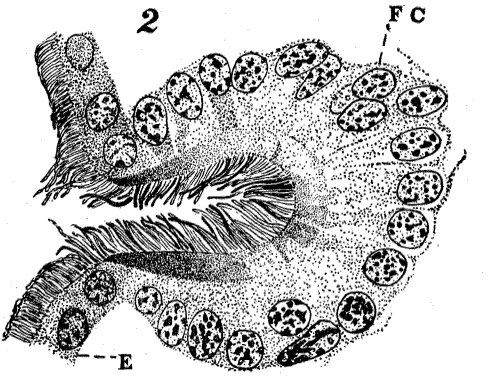
Fig. 1. Sexually mature specimen seen from left side. The ovary with oöcytes and oögonia shown lying by the left side of enteron. *V*, vagina; *M*, mouth leading into pharynx; *CP*, ciliated pit. Scale, 1 millimeter equals 7.5 micra.

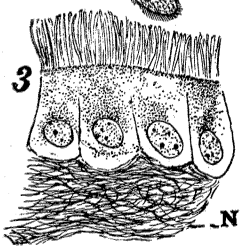
Fig. 2. Ciliated pit arising from the epidermis. *E*, epidermis; *FC*, cells at fundus of pit. ×1,500.

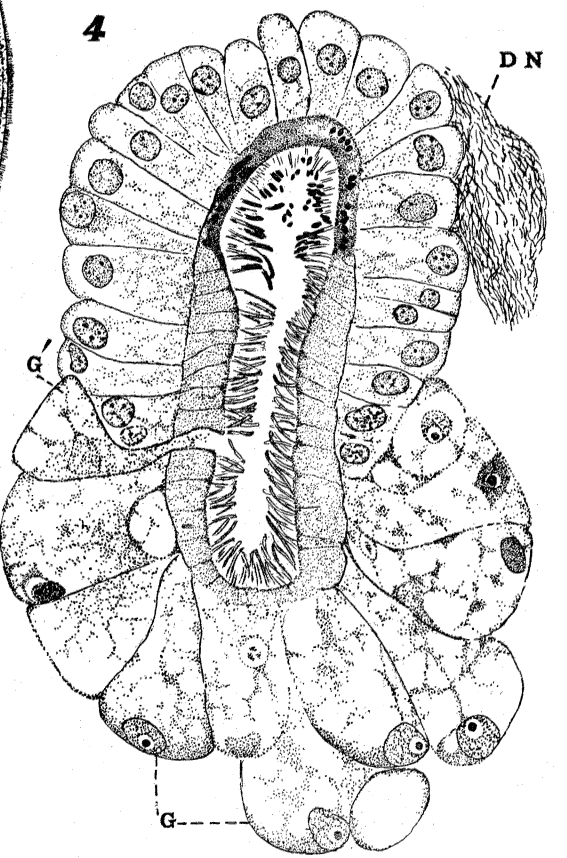
Fig. 3. Gustatory epithelium of pharynx. The basal ends of the cells lying directly upon the nerve commissure. *N*, part of the nerve commissure. ×1,500.

Fig. 4. Sagittal section of ciliated pit. *DN*, dorsal, lateral nerve upon which the basal ends of the sensory cells rest; *G*, glandular cells of fundus; *G'*, a glandular cell which presents a duct leading into lumen of the pit. ×1,500.









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